THE REGULATION OF LITHIUM IN CEREBROSPINAL FLUID BY THE IN SITU ISOLATED CHOROID PLEXUS OF THE CAT

by

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SUPERVISORY COMMITTEE APPROVAL

of a dissertation submitted by

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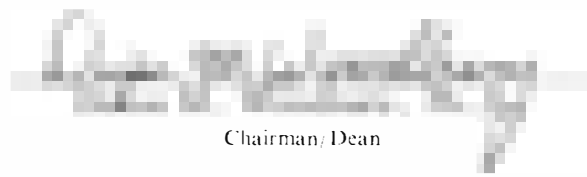

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ABSTRACTS

It is well established that the choroid plexus is a major site of c.s.f. production and for regulating the composition of the c.s.f. The in situ isolated choroid plexus preparation provides a unique system with which to study the role of the choroid plexus in the regulation of ion transport from blood to c.s.f. and vice versa. The concentration of lithium in the interstitial fluid of brain is known to affect the function of the brain. Measurement of the c.s.f. concentration of lithium serves as a gross reflection of the lithium concentration in the extracellular fluid of the C.N.S. The role of the choroid plexus in regulation of lithium concentration in the c.s.f. with and without alterations in c.s.f. and plasma levels of this ion was studied. The change in concentration of lithium in the chamber fluid was about 0.58 m-equiv/l. for each 1 m-equiv/l. change in plasma concentration of lithium in response to the infusion of a 154 mM LiCl solution. The lithium concentration in chamber fluid was maintained at 53% to 60% of plasma levels when the plasma lithium concentrations varied over the therapeutic and the toxic range in man. The concentration of lithium reached a

steady state in plasma and in c.s.f. at about 90 min after the start of a constant intravenous infusion of LiCl solution. This shows that the slow onset of action of lithium in the treatment of manic depression is not due to the inability of lithium to cross the blood-c.s.f. barrier. The concentration of lithium in the chamber fluid decreased during the 30 min collection period when either low or high lithium in artificial c.s.f. was added to the chamber. The magnitude of the decrease is independent of the direction of the gradient between plasma and c.s.f. over the range studied. This indicates that dilution by newly formed fluid is a major factor in reducing the concentration of lithium in the chamber. It is reduced secondarily by passive diffusion. The change in the concentration of potassium on the c.s.f. side of the choroid plexus induced appropriate alterations in the new c.s.f. potassium to bring the concentration of potassium in the chamber fluid toward a normal value. Lithium appears to be transported by the same system that regulates c.s.f. potassium. However, the system is much more selective for potassium. These results support the concept that lithium is transported to a limited extent by sodium pumps and also diffuses passively.

The mechanisms underlying the therapeutic usefulness of lithium in mania are not known. The in situ isolated choroid plexus in cats was used to investigate the effect of varying the lithium

concentration in plasma and in c.s.f. on the concentration of electrolytes in the c.s.f. The concentration of magnesium in plasma was 2.7 m-equiv/l. or more, while chamber fluid magnesium was not affected. The chamber fluid to plasma ratio of ^{22}Na is greater than unity after a 90 min infusion of $^{22}NaCl$ solution. The total amount of ^{22}Na in the chamber fluid significantly increased when lithium (1.5 m-equiv/l.) or high-potassium (6.6 m-equiv/l.) artificial c.s.f. was added to the chamber; there is no difference in ^{22}Na activity between the control and the experimental group in which 50 µl of normal artificial c.s.f. was added to the chamber. Ouabain (10^{-3} M) significantly inhibited the stimulatory effect of lithium or high-potassium on the rate of ^{22}Na transport from blood to c.s.f. and also reduced the rate of c.s.f. secretion. The entry of ^{22}Na into the chamber was not affected by plasma lithium levels corresponding to the subtherapeutic to therapeutic levels in man. The present experiments suggest that lithium is transported by systems that normally transport sodium into the c.s.f. to produce the fluid and transport potassium out of c.s.f. in exchange for sodium. There is also a significant diffusional component.

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PART ONE

THE REGULATION OF CEREBROSPINAL FLUID LITHIUM BY THE ISOLATED IN SITU CHOROID PLEXUS OF THE CAT

INTRODUCTION

Cade (1949) reported that lithium salts given to ten manic patients resulted in uniform improvement. Since then, investigators have extensively studied the pharmacological and biological activity of lithium. If serum lithium is to be maintained at levels sufficient to provide therapeutic concentrations in the C.N.S. for the treatment of manic-depression and other mental illness, information on the relation between plasma levels and c.s.f. concentrations of this ion is necessary. Schou (1958) reported that lithium passes into a variety of tissues from the blood and that the entry rates vary in different tissues and organs; the mechanisms governing its entry into the C.N.S. were not defined. Because little definitive information is available concerning the entry of lithium into the C.N.S. data concerning lithium movements across other biological membranes are of interest. Experiments on lithium flux in frog skin, frog muscle, erythrocytes and mammalian nerve tissue, i.e., non-myelinated C-fibers, have been performed. The flux of lithium into frog muscle is similar to the passive influx rate of sodium (Keynes & Swan, 1959). Also, lithium and sodium permeabilities are of the same order of magnitude for erythrocytes (Maizels, 1954). In both tissues, there appears to be no active influx of lithium. However, recently Meltzer, Rosoff, Kassir & Fieve. (1976) and Haas, Schooler, & Tosteson. (1975) found that there is an active efflux of lithium from erythrocytes. There is some evidence for active transport of lithium in certain epi-

helial membranes. Herrera, Egea, & Herrera. (1971) demonstrated that lithium influx across toad bladder was inhibited by ouabain. Since ouabain is an inhibitor of the active sodium transport mechanism, the authors suggested that lithium moves across the bladder, at least in part, by the active sodium transport pathway. Zerahn (1955) demonstrated that lithium and sodium compete equally well for the same transport pathway in frog skin. Furthermore, he established that frog skin transports lithium against both electrical and chemical gradients and concluded that the transport process was active. Recently, Candia & Chiarandini (1973) confirmed that under certain conditions lithium is actively transported by frog skin. These workers concluded that lithium ions can both inhibit the sodium pump and be actively transported by it.

Although the above data indicate that certain epithelial membranes are capable of active lithium transport, there is no indication that the same is true of the epithelial membrane separating blood from c.s.f. and brain tissue. Wright (1972) and Prockop & Marcus (1972) used frog or rabbit choroid plexus incubated in artificial c.s.f. containing both lithium and other cations to demonstrate that the T:M (tissue:medium) ratio is less than one and that this ratio is compatible with lithium movement into the tissue by simple diffusion. Prockop & Marcus (1972) performed ventriculocisternal perfusion studies in dogs and observed that total lithium clearance could be accounted for by bulk flow and simple diffusion. Simultaneously determined lithium and creatinine clearances were similar. However, these studies did not establish the mechanism of lithium entry from blood to c.s.f. and vice versa. Thus, the exact mechanisms controlling the

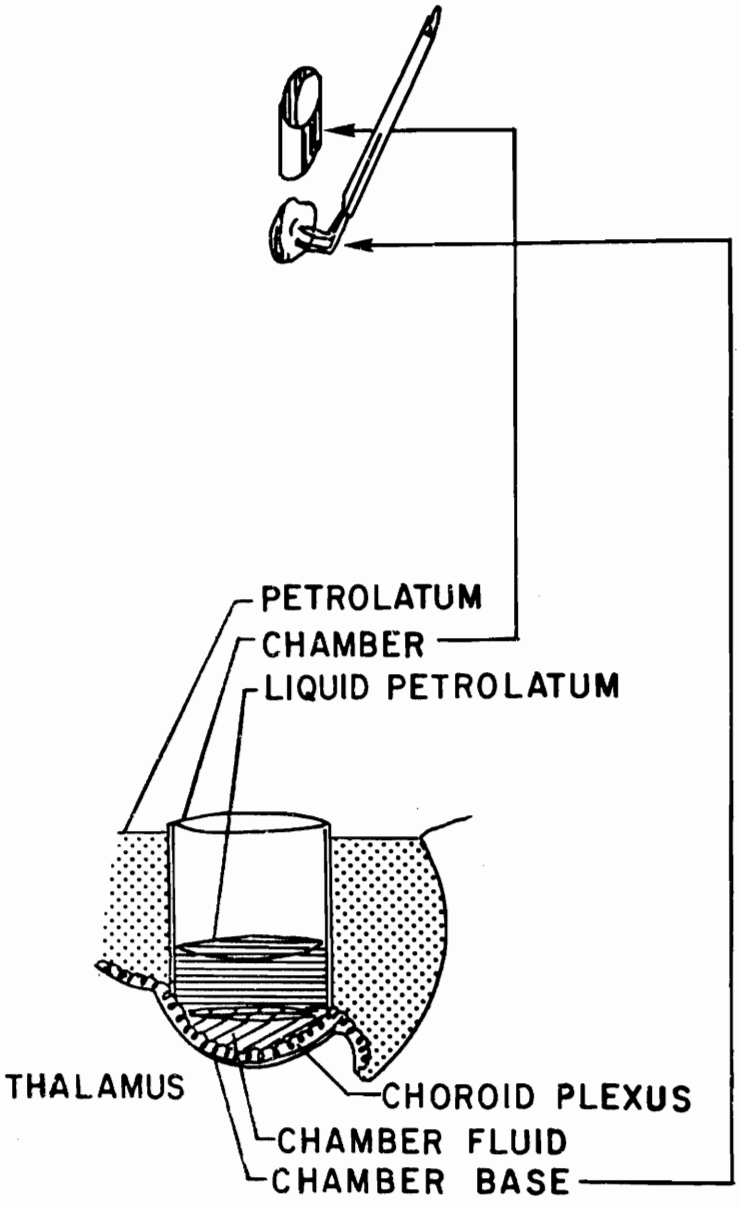
movement of lithium between blood and c.s.f. are not established. The present study was undertaken to elucidate the role of the choroid plexus in regulating the c.s.f. concentration of lithium with and without alterations in c.s.f. and plasma levels of this ion.

METHODS and MATERIALS

Experimental preparation. Adult cats of either sex, body weight 2.5 to 4.5 kg, were anesthetized with sodium pentobarbital, 30 mg/kg, given intraperitoneally. A tracheotomy was performed and the animal was maintained on artificial respiration throughout the experiment. The femoral artery was cannulated for monitoring blood pressure and for collecting blood samples. The femoral vein was cannulated to permit intravenous administration of supplemental anesthetic and infusion of 154 mM (isotonic) LiCl solution. A Harvard infusion pump was used to infuse the LiCl when constant levels of lithium in plasma were desired. Body temperature was maintained near 37°C with an electric heating pad. A part of the choroid plexus in the left lateral ventricle was isolated in a chamber (Fig.1) in situ as described by Miner & Reed (1972). In brief, the method requires an extensive unilateral craniotomy, removal of the cerebral cortex overlying the left lateral ventricle and exposure of the plexus within the lateral ventricle. An appropriate segment of the plexus is then separated from its underlying connective tissue attachments and isolated within a chamber with the blood and nerve supply intact.

Collection of chamber fluid. After completion of the isolation procedure all the fluid in the chamber was removed. Fluid secreted by the choroid plexus was allowed to collect in the chamber

Fig. 1. Diagram of the chamber used.


PETROLATUM
CHAMBER
LIQUID PETROLATUM
THALAMUS
CHOROID PLEXUS
CHAMBER FLUID
CHAMBER BASE

under a layer of liquid petrolatum that was added to eliminate fluid evaporation. In some experiments 50 µl of artificial c.s.f. (described below) was added to the chamber at the start of the collection period. Each collection period was 30 min. An arterial blood sample (0.5 ml) was obtained at the midpoint of each collection period.

Artificial cerebrospinal fluids. The artificial c.s.f. described by Merlis (1940), or with slight modifications, was used in all experiments. The low-lithium and high-lithium fluids were prepared by interchanging NaCl and LiCl in the artificial c.s.f. so that the final lithium concentrations were 1.8 m-equiv/l. or 4.5 m-equiv/l., respectively.

Analysiss. A protein-free plasma supernatant was prepared by adding 4 ml of 0.5 N HNO_3 to 0.2 ml of plasma which was then centrifuged at 3000 r.p.m. Ten µl of chamber fluid or 2 ml of the plasma supernatant were diluted 1:1000 or 1:2, respectively, with 0.9% NaCl solution for determining the concentration of lithium. Lithium was determined with a model 305A Perkin-Elmer Atomic Absorption Spectrophotometer. Protein concentrations were determined by the method of Pesce & Strande (1973) on 20 µl samples of the chamber fluid and a comparable volume of a 1:20 dilution of whole plasma.

Calculations. The concentration of lithium in the newly formed c.s.f. was calculated by subtracting the amount of lithium initially added to the chamber in the artificial c.s.f. from the total amount in the chamber at the end of 30 min and dividing by the volume of newly formed fluid. The formula used is:

$$\text{New c.s.f. } (X) = \frac{(X)_{cf} V_{cf} - (X)_a V_a}{V_{cf} - V_a}$$

Where (X) is the concentration of the substance in the fluid, V is the fluid volume and the subscripts "cf" and "a" refer to chamber fluid and added artificial c.s.f., respectively. It is assumed that any change in chamber fluid concentration was due to the addition of the volume of newly formed fluid of the calculated composition. This assumption may not be absolutely correct but it is not important for the purposes of this presentation whether the change in the quantity of the substance in the chamber is expressed as a flux or as a concentration in the new fluid. This value will be negative if lithium is lost from the chamber.

Experimental procedure. Three series of experiments were done.

1. The plasma concentration of lithium was varied by the infusion of 154 mM LiCl solution. Blood and chamber fluid were collected at 30 min intervals.

2. Artificial c.s.f. that contained lithium was added to the chamber before and after the intravenous infusion of lithium. In all cases where lithium was infused the plasma lithium concentration was maintained higher than that in the chamber. Chamber fluid was removed. every 30 min.

3. Three concentrations of lithium and potassium were added to the chamber, namely, lithium:potassium=2.6:1.7; lithium:potassium=5.2:3.5; lithium:potassium=10.2:6.7 m-equiv/l. which provided the same lithium:potassium ratio. Blood lithium was assumed to be negligibly small. Chamber fluid was collected every 30 min.

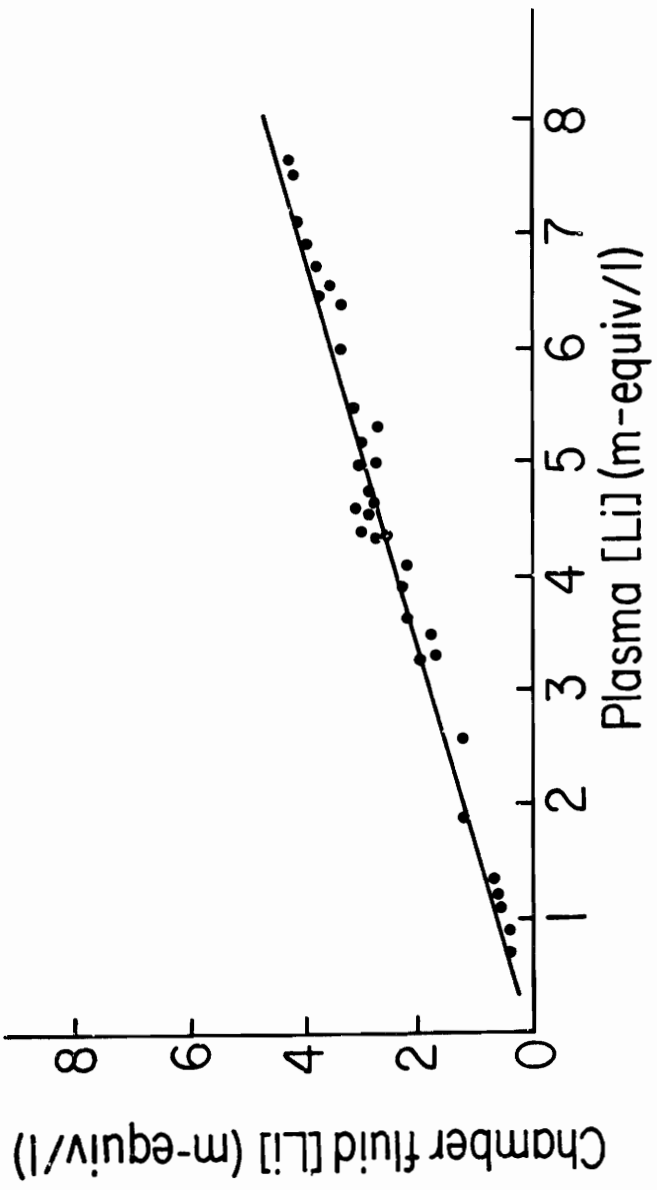
RESULTS

The range of fluid accumulation rates in the chamber was 0.6 to 2.7 µl/min. The wet weight of the choroid plexus within the chamber ranged from 2.2 to 5.6 mg. On the basis of the average rate of fluid accumulation and the average wet weight of plexus in the chamber, the rate of c.s.f. production by the plexus was about 0.4 to 0.5 µl/min/mg as has been previously reported (Miner & Reed, 1972; Husted & Reed, 1976; Reed & Yen, 1978). The rate of fluid secretion was unaffected by the initial addition of 50 µl of the artificial c.s.f. into the chamber.

The mean protein content of new c.s.f. was 0.69 g/100 ml (range 0.06 to 1.34 g/100 ml). These protein values are similar to those previously reported in studies in which the same techniques were used (Miner & Reed, 1972, Husted & Reed, 1976). The protein concentration of the c.s.f. remained reasonably constant throughout any one experiment although the first chamber fluid sample often had a slightly higher protein content than subsequent ones.

The relationship of chamber fluid lithium concnetration to that in plasma, as measured in the first series of experiment, is shown in Fig. 2. Each point on the graph represents the value of one 30-min collection of chamber fluid and its corresponding plasma value. Usually 6 to 8 collections were made from each animal. The range of lithium concentrations in plasma was obtained by infusion of lithium at a constant rate into animals of varying weights. The line was calculat-

Fig. 2. The relationship of chamber fluid lithium concentration to that in plasma after intravenous infusion of 154 mM LiCl solution. Each point represents the value of one 30 min collection of chamber fluid and its corresponding plasma level (n=5). The line was calculated by linear regression analysis of all the data points and has a slope of 0.58 ± 0.03)r=0.98).


Chamber fluid [Li] (m-equiv/l)
0
2
4
6
8
1
2
3
4
5
6
7
8
Plasma [Li] (m-equiv/l)

ed by linear regression analysis of all the data points and has a slope of 0.58 ± 0.03 (r=0.98).

A plot of the ratio of lithium in the chamber fluid to that in the plasma as a function of time after the start of the intravenous infusion of LiCl is shown in Fig. 3. The c.s.f.-to-plasma ratio varied from 0.53 to 0.60 when plasma levels ranged from 0.7 to 1.5 m-equiv/l.; these concentrations are in the therapeutic range in man. It is apparent that the c.s.f.-to-plasma ratio is less than one and that the ratio remains constant after the first collection period.

The concentration of lithium in plasma and c.s.f. is plotted versus time after the start of an i.v. infusion in Fig. 4. The concentration of lithium in plasma and c.s.f. was in a steady state at about 90 min.

The effect of the plasma lithium concentration on the change in chamber fluid lithium as measured in the second series of experiments is shown in Fig. 5. Two groups of experiments were performed. Either high-lithium or low-lithium artificial c.s.f. was added to the chamber with no lithium in the plasma or with the plasma lithium maintained higher than that in the chamber. The dashed lines represent the concentration of lithium in the artificial c.s.f. solutions that were added to the chamber initially. Each point on the graph represents the mean concentration of lithium in the chamber fluid at the end of a 30 min collection period. Usually at least two 30 min collections were obtained from each animal with each added artificial c.s.f.. When high (4.5 m-equiv/l.) or low (1.8 m-equiv/l.) lithium artificial c.s.f. was added to the chamber with no lithium in the blood, the lithium concentration in the chamber at the end of the

Fig. 3. The chamber fluid to plasma ratio of lithium as a function of time after the start of an intravenous infusion of 154mM (isotonic) LiCl solution. The bar for each point represents the s.e.m. (n=5).

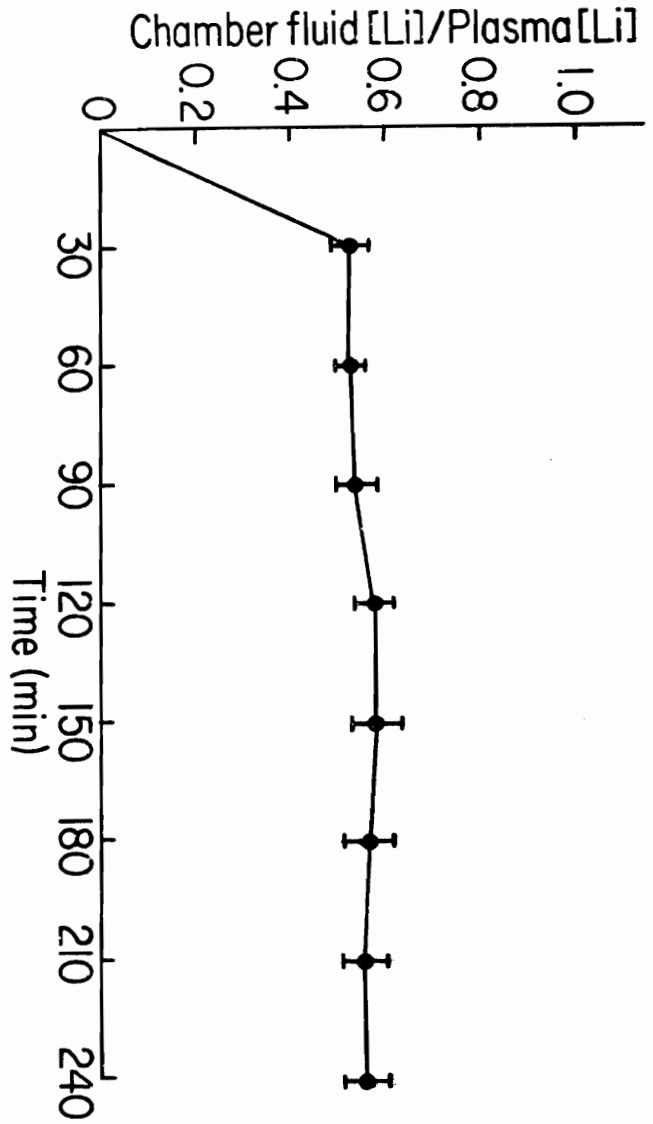

Chamber fluid [Li]/Plasma [Li]
0
0.2
0.4
0.6
0.8
1.0
30
60
90
120
150
180
210
240
Time (min)

Fig. 4. The concentration of lithium in plasma and c.s.f. as a function of time after intravenous infusion of 154 mM (isotonic) LiCl solution. The bar for each point represents the s.e.m. (n=5).

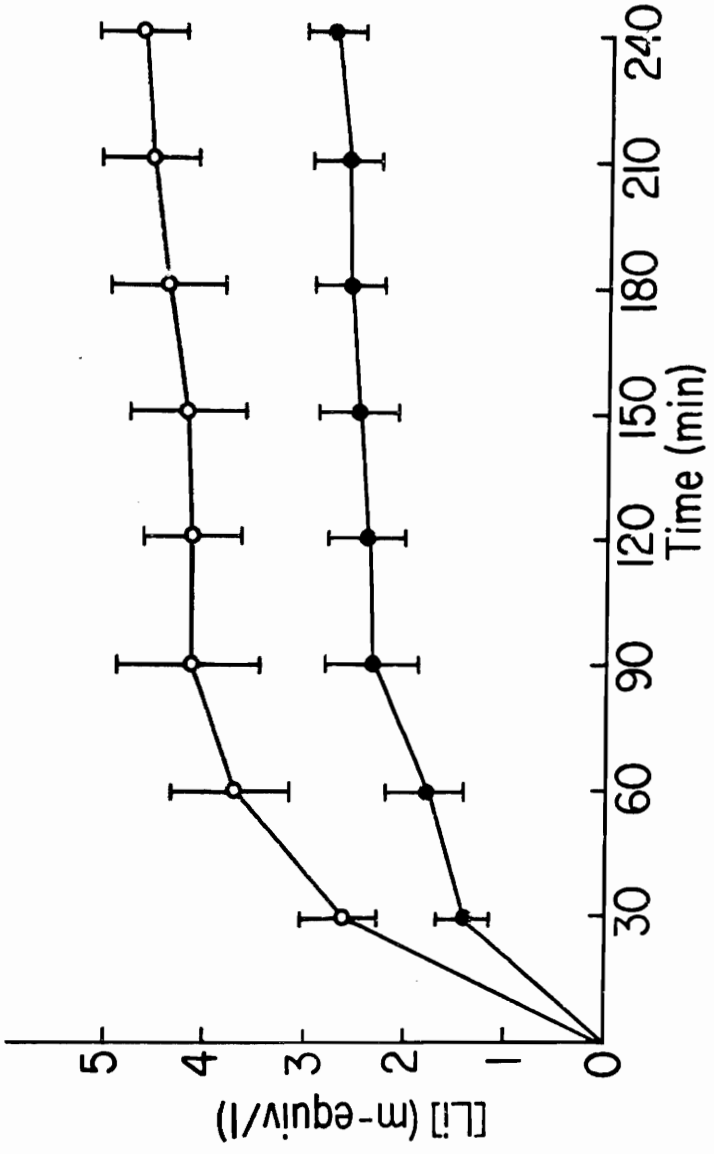
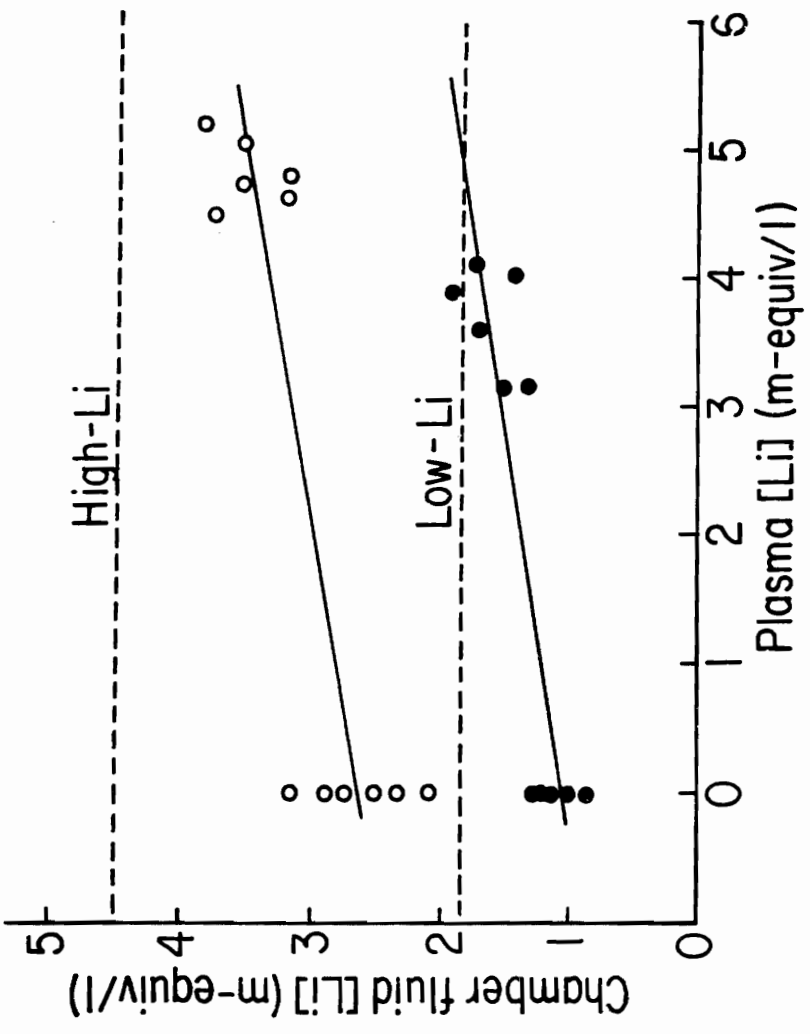

[Li] (m-equiv/l)
0
1
2
3
4
5
30
60
90
120
150
180
210
240
Time (min)

Fig. 5. The effect of the plasma lithium concentration on the change in chamber fluid lithium. The results of two groups of experiments are shown. Either high (4.5 m-equiv/1.) or low (1.8 m-equiv/1.) artificial c.s.f. was added to the chamber with no lithium in the plasma or with the plasma lithium maintained higher than that in the chamber. The dashed lines represent the concentration of lithium in the artificial c.s.f. that was added to the chamber initially. Each point represents the mean concentration of lithium in the chamber fluid at the end of a 30 min collection period. (n=6).


High-Li
Low-Li
Chamber fluid [Li] (m-equiv/l)
Plasma [Li] (m-equiv/l)
0
1
2
3
4
5
6

collection period had decreased to 2.6 and 1.0 m-equiv/1., respectively. However, when these experiments were repeated with the plasma lithium concentration greater than that of the artificial c.s.f. initially, the chamber fluid concentration decreased only to 3.5 and 1.7 m-equiv/1., respectively. The change in the total amount of lithium in the chamber was calcualted from the data in Fig. 5 and is shown in Fig.6. When 50 µl of low (1.8 m-equiv/1.) or high (4.5 m-equiv/1.) artificial c.s.f. was added into the chamber, with no lithium in the blood, the net change in the amount of lithium in the chamber during a 30 min collection was -15.2×10^{-6} and -33.2×10^{-6} m-equiv (columns A and C), respectively. The negative values indicate that lithium was removed from the chamber fluid during the collection period. In experiments in which the plasma lithium concentration was maintained greater than that in the fluid initially added to the chamber, the results were 33×10^{-6} and 34.9×10^{-6} m-equiv/1. (columns B and D), respectively.

Three different artificial c.s.f. solutions were used in the thrid series of experiments, namely, lithium:potassium=2.6:1.7; lithium:potassium=5.2:3.5; lithium:potassium=10.2:6.7 m-equiv/1. The lithium and potassium concentrations were different but the lithium:potassium ratios were the same, and no lithium was infused into the blood. The concentration of lithium and potassium in the chamber fluid was determined after 30 min. Each solution with a different concentration of lithium:potassium was added randomly to the chamber in each animal. The results are shown in the Table 1. The lithium and potassium concentrations in the chamber fluid at the ends of the collection periods were significantly different from the initial values when

Fig. 6. The change in the total amount of lithium in the chamber after 30 min. Columns A and C represent the net loss of lithium from the chamber when the plasma lithium concentration was zero. Columns B and D represent the net gain of lithium into the chamber fluid when the plasma lithium concentration was maintained greater than that in the chamber. The values were derived from the data in Fig. 5 (n=6).

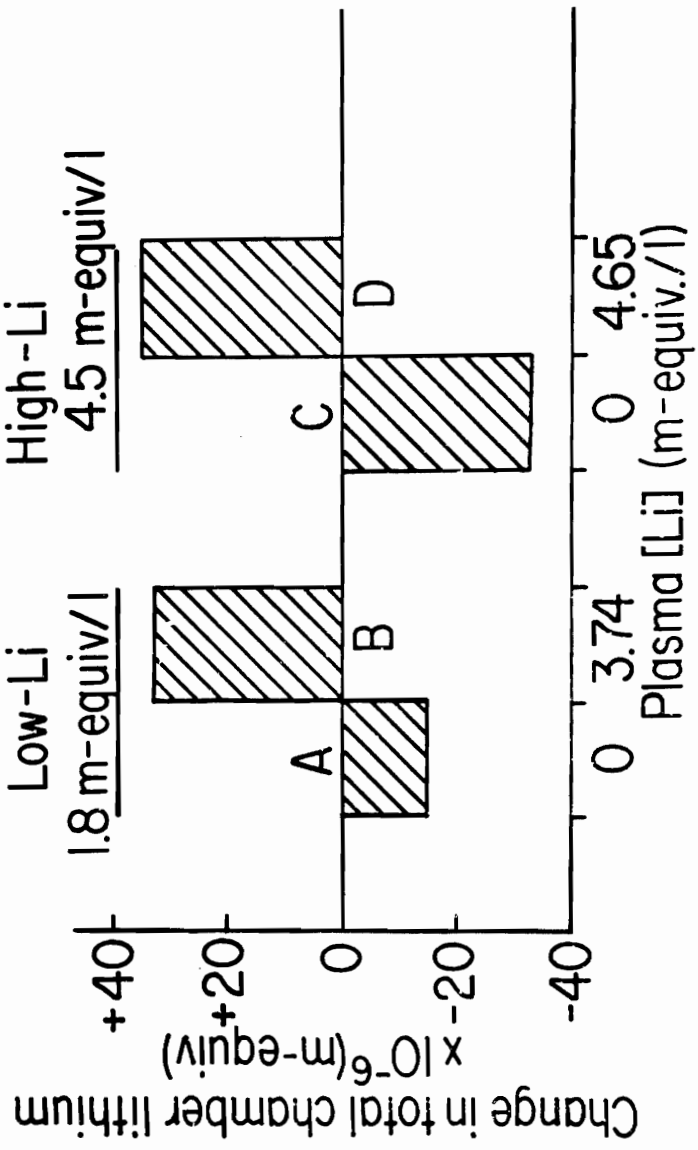

Change in total chamber lithium
x 10⁻⁶(m-equiv)
+40
+20
0
-20
-40
Low-Li
1.8 m-equiv/l
High-Li
4.5 m-equiv/l
A
B
C
D
0
3.74
0
4.65
Plasma [Li] (m-equiv./l)

Table 1. The concentration of lithium and potassium in the chamber fluid initially and after the 30 min collection periods.

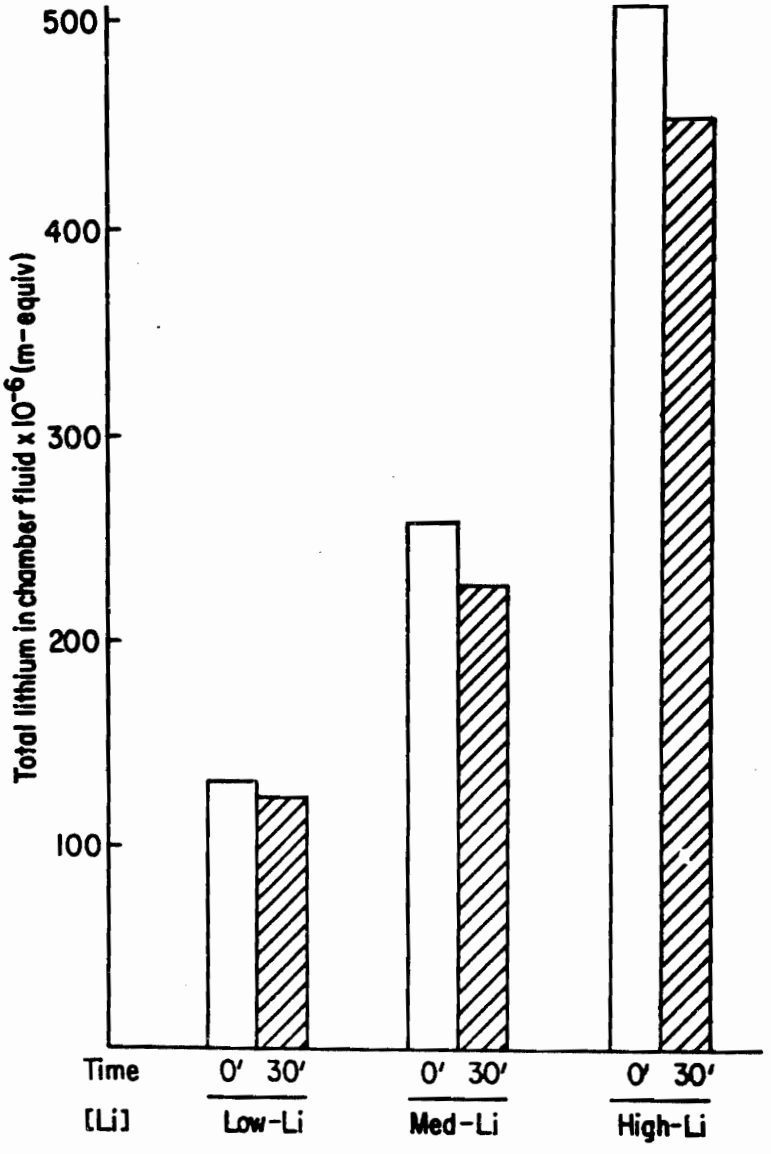
CATION / TIME / ION RATIO / CONCENTRATION	0 MIN (initial)	30 MIN (final)	II/I x 100%	
	Chamber Con'c (I) Li:K (m-equiv/l)	Chamber Con'c (II) Li:K (m-equiv/l)	Li	K
Low	2.6 : 1.7	1.6 ± 0.1 : 2.2 ± 0.1	61	130
Medium	5.2 : 3.5	2.9 ± 0.2 : 2.8 ± 0.1	56	80
High	10.2 : 6.7	5.9 ± 0.2 : 4.3 ± 0.2	58	64

Note: The II/I ratio represents the final concentration of the ion in the chamber fluid as a percent of the initial value.

either low, medium or high lithium-potassium was added (P 0.01). The ratio of lithium in the chamber fluid at the end of the 30 min collection to that in the fluid added to the chamber initially was about the same (0.6) with each of the combinations used. However, in contrast to lithium, the ratio for potassium with low-potassium artificial c.s.f. was 1.3; with medium-potassium artificial c.s.f., 0.8; and with high-potassium artificial c.s.f., 0.64.

The total amounts of lithium in the chamber at the beginning and the end of the collection periods are shown in Fig. 7. The total amount of lithium in the chamber decreased after adding the three different concentrations of lithium and potassium in artificial c.s.f.s. The magnitude of the decrease is proportional to the concentration of the lithium in the artificial c.s.f. added to the chamber.

Fig. 7. The total amount of lithium in the chamber at the beginning and the end of a collection period after adding three different solution with the same ratio of lithium and potassium. The values are derived from the data in Table 1. (n=6).


500
400
300
200
100
Total lithium in chamber fluid x 10^{-6} (m-equiv)
Time
0′ 30′
0′ 30′
0′ 30′
[Li]
Low-Li
Med-Li
High-Li

DISCUSSION

The present study was intended to yield evidence about the role of the choroid plexus in regulating the lithium concentration in the c.s.f. Physiological and pharmacological evidence have demonstrated that the choroid plexus plays a major role in electrolyte homeostasis and production of c.s.f. It is well established that the composition of brain interstitial fluid is close to that of c.s.f.

In the first series of experiments it was shown that the concentration of lithium in newly formed chamber fluid is less than that in plasma after intravenous infusion of LiCl solution. It is apparent that the lithium concentration in chamber fluid has a linear correlation with the plasma levels. These data are consistent with the report by Wraae (1978) that a high correlation exists between the steady state concentration of lithium in human serum and that in c.s.f.

The c.s.f. /plasma ratio of 0.58 may be due to at least two mechanisms, passive dissusion and/or active transport. If it is assumed that lithium moves passively and freely across the choroid plexus and is in equilibrium with the membrane potential, then the distribution of lithium between blood and c.s.f. may be predicted by the Nernst equation. A potential difference of + 2-5 mV between blood and c.s.f. (c.s.f. positive) determined by Husted & Reed (1977) using the same preparation as in the present study. Calculation of the distribution of lithium between c.s.f. and plasma with a potential difference of +2 mV gives a value of 0.93 (93:100) and with a potential

difference of +5 mV a value of 0.83 (83:100). The slope of the line describing the relationship between lithium in the c.s.f. (chamber fluid) and in the plasma is 0.58 (Fig 2). This value is significantly different from those predicted above on the basis of purely passive distribution of lithium. Assuming that the potential difference across the choroid plexus remained within the range of +2-5 mV during the experiments, the data indicate that lithium is not in equilibrium with the membrane potential. There are two possible explanations for a slope of less than one (0.58). If the passive diffusion from blood to c.s.f. is slower than the rate of c.s.f. secretion and/or if lithium is actively transported with the secreted fluid via the sodium pump but the selectivity for sodium is greater than for lithium, then the end result is that the ratio should be less than one. If sodium is transported across the choroid plexus into the c.s.f. and assuming that there is no selectivity between lithium and sodium by the sodium pump, lithium would be expected to appear in the c.s.f. in the same proportion to sodium as existed in the plasma. In this case the slope of the line in Fig. 2 should have been slightly greater than one yielding a lithium concentration higher in the c.s.f. than in the plasma. However, the observed slope is less than one. This suggests that if lithium is transported by the sodium pump, it is more selective for sodium than for lithium. Keynes & Swan (1959) found that the rate of transport of lithium out of frog muscle is 1/10 to 1/25 that of sodium. Given a similar relationship for these ions in the choroid plexus, it follows that the relative amount of lithium pumped into the c.s.f. via the sodium pump by the choroid plexus would be less than that of sodium. Also, it is well established that the movement of

sodium across the choroid plexus is highly correlated with the formation of new c.s.f. Thus, the limited transport of lithium via the sodium pump across the choroid plexus could produce c.s.f. with a lithium concentration less than that in plasma and serve to explain a lower concentration of lithium in the c.s.f. than in the plasma.

The concentration of lithium in newly formed c.s.f. was maintained at 0.53 to 0.60 of the plasma levels as shown in Fig. 3. Several different investigators have reported values for the c.s.f. to plasma ratio for lithium, the range varies from 0.25 to 0.67 (Hanlon, Romaine, Gilroy & Deitrick, 1949; Schou, Juel-Nielson, Stromgreen & Voldby, 1954; Baker & Winokur, 1966; Platman Rohrlich & Fieve, 1968; Gershon, 1970 ;Watanabe, Taguchi, Ebara, Iguchi & Otsuki, 1973. The reasons for such a large variation in lithium ratios may be related to differences in the species studied, the length of the experiments (acute or chronic) and in the experimental preparations and techniques.

The onset of action of lithium in the treatment of mania is relatively slow, and a full effect cannot be expected until after 6 to 10 days of treatment (Anderson & Prockop, 1975; Baer, 1976; Amdisen, 1977; Mason, McGueen, Keary & James, 1978). The data shown in Fig. 4. indicate that lithium reached a steady state in plasma and in c.s.f.in this preparation in about 90 min. From these results, it can be inferred that the slow onset of action of lithium is not due to its slow rate of entry into the C.N.S. It is well established that the c.s.f. is in equilibrium with the ECF of brain so it would be expected that lithium would reach a therapeutic concentration promptly at the neuron. However, a much longer time may be required for lithium to produce its effect if the effect is due to depletion or synthesis of the substances

which ultimately produces the effects attributed to lithium. In any case, difficulty in crossing the blood-c.s.f. barrier by lithium appears entirely inadequate to explain its delayed action.

From the data shown in Fig. 5 it can be seen that the concentration of lithium in the chamber decreased during the 30 min collection periods when either low or high lithium artificial c.s.f. was added to the chamber whether the plasma lithium concentration was zero or greater than that in the chamber. The lower concentration of lithium in the chamber fluid may be explained in two possible ways:
(1) lithium was moved out of the chamber either by passive dissusion or by active transport. (2) Lithium may have been diluted by newly formed fluid with a lower lithium concentration. If it is assumed that lithium diffused passively across the choroid plexus, the change in the lithium concentration of the chamber fluid would be expected to be greater with a high concentration gradient than with a low one. However, in the present study the concentration of lithium in the chamber after the 30 min collection period was about 45% of that of the low or high-lithium artificial c.s.f. initially added to the chamber when no lithium was present in plasma. However, it is impossible to discount completely any contribution of passive diffusion in this experiment since the data in Fig. 6 show that the net loss of lithium from the chamber is proportional to the concentration gradient when the plasma concentration is zero. This implies that a passive diffusion component, though small, was present but was obscured by dilution when viewed as a change in concentration in Fig. 5. When the concentration gradient was in the opposite direction as also shown in Fig. 6, namely, from plasma to chamber fluid, there was a net gain of lithium in the chamber which was not different when low or high lithium was added to the chamber. Superfi-

cially this may seem inconsistent with the data shown in Fig.5,i.e., a decrease in chamber fluid lithium concentration regardless of the direction of the gradient. However, when the gradient is from plasma to chamber fluid there is a net gain in total lithium in the chamber with a concomitant decrease in lithium concentration due to dilution of the initial fluid by newly formed c.s.f. which decreased the total concentration. Total lithium was increased due to the addition of the lithium in the newly formed c.s.f. as shown by Fig. 3. On the basis of these data it is suggested that the dilution by newly formed fluid is major factor in maintaining the low concentration of lithium in the chamber with passive diffusion exerting a quantitatively smaller effect.

given the amount of lithium in the chamber initially as in Fig. 5, the resultant concentration after 30 min can be closely predicted form the data in Fig. 2 which gives the concentration in the newly formed c.s.f.. Therefore, it is possible to predict the lithium concentration in the chamber fluid after 30 min when given the plasma lithium level and the initial lithium concentration in the chamber fluid.

The third series of experiments was designed to ascertain whether lithium was transported by the transport system that regulates potassium. Three different artificial c.s.f. solutions with the same ratio of potassium to lithium were added to the chamber. The data in table 1 show that the change in the concentration of lithium is different from that of potassium. Husted & Reed (1976) demonstrated that the potassium concentration in c.s.f. is actively regulated by the choroid plexus, which senses the alterations in potassium that occur in

c.s.f.. This was also observed in the present study. The data in Fig. 7 show that the total amount of lithium in the chamber decreased after adding the three different concentrations of lithium. The magnitude of the decrease was proportional to the concentration gradient between chamber fluid and plasma. Thus, passive diffusion and/or active transport processes probably serve as the mechanisms involved in the regulation of lithium in chamber fluid. The concentration is further reduced by dilution with newly formed fluid. Whittam (1962) has noted that lithium ion is able to replace both external potassium and internal sodium ions and is unlike other alkali metals in that it possesses the ability to stimulate ATPase activity from both sides of the erythrocyte membrane. Beauge (1975) reported that in frog muscle, potassium showed a higher affinity than lithium for the pump site. It is also demonstrated in the following paper that the rate of ^{22}Na transport from blood to c.s.f. increases after adding lithium into the chamber. This supports the suggestion that lithium is transported across the choroid plexus to the blood via the same transport system as potassium however, the data in Table 1 show that the percent change in lithium is the same after the addition of each of the three different concentrations of lithium to the chamber. This suggests that the lithium contration in c.s.f. is mainly regulated by the newly formed fluid with possibly some active transport via a potassium transport system. In view of all of the experimental results, it appears that the transport of lithium between blood and c.s.f. is by passive diffusion and some active transport via the sodium pumps responsible for c.s.f. secretion and for sodium-potassium exchange.

REFERENCES

Amdisen, A. (1977). Serum level monitoring and clinical pharmacokinetics of lithium. Clin. Pharmacokinetics 2, 73-92.

Anderson, D.K. & Prockop, L.D. (1975). Lithium absorption and distribution in body tissues. In Lithium Research and Therapy. ed., Johnson, F.N. pp. 267-278, Academic Press: New York.

Baer, L. (1976). Pharmacology-lithium absorption, distribution, renal handling, and effect on body electrolytes. In Lithium-Its Role in Psychiatric Research and Treatment. ed., Gershon, S. & Shopsin, B. pp. 33-37, Plenum Press: New York.

Baker, M.A. & Winokur, G. (1966). Cerebrospinal fluid lithium in manic illness. Brit. J. Psychiat. 112, 163-165.

Beauge, L. (1975). The interaction of lithium ions with the sodium potassium pump in frog skeletal muscle. J. Physiol. 246, 397-420.

Cade, J.F.J. (1949). Lithium salts in the treatment of psychiatric excitement. Med. J. Aust. 36, 349-352.

Candia, O.A. & Chiarandini, D.J. (1973). Transport of lithium and rectification by frog skin. Biochem. Biophys. Acta. 307, 578-589.

Gershon, S. (1970). Lithium in mania. Clin. Pharmac. Ther. 11, 168-187.

Haas, M., Schooler, J., & Tosteson, D.C. (1975). Coupling of lithium to sodium transport in human red cells. Nature 258, 425-427.

Hanlon, L.W., Romaine, M., Gilroy, F.L. & Deitrick, J.E. (1949). Lithium chloride as a substitute for sodium chloride in the diet. J.Am. Med. Assoc. 139, 688-692.

Herrera, F.C., Egea, R., & Herrera, A.M. (1971). Movement of lithium across toad urinary bladder. Am. J. Physiol. 220, 1501-1508.

Husted, R.F. & Reed, D.J. (1976). Regulation of cerebrospinal fluid potassium by the cat choroid plexus. J. Physiol. 259, 213-221.

Husted, R.F. & Reed, D.J. (1977). Regulation of cerebrospinal fluid biocarbonate by the cat choroid plexus. J. Physiol. 267, 411-428.

Keynes, R.D. & Swan, R.C. (1959). The permeability of frog muscle fibers to lithium ions. J. Physiol. 47, 626-638.

Maizels, M. (1954). Active cation transport in erythrocytes. Symp. Soc. Exp. Biol. Med. 8, 202-227.

Mason, R.W., McQueen, E.G., Keary, P.J., & James, N. Mcl. (1978). Pharmacokinetics of lithium: elimination, half-time, renal clearance and apparent volume of distribution in schizophrenia. Clin. Pharmacokinetics 3, 241-246.

Meltzer, H.L., Rosoff, C.J., Kassir, S., & Fieve, R.R. (1976). Active efflux of lithium from erythrocyte of manic depressive subjects. Life Sci. 19, 371-380.

Merlis, J.K. (1940). The effect of change in the calcium content of cerebrospinal fluid on spinal reflex activity in the dog. Am. J. Physiol. 131, 67-72.

Miner, L.C. & Reed, D.J. (1972). Composition of fluid obtained from choroid plexus tissue isolated in a chamber in situ. J. Physiol. 227, 127-139.

Pesce, M.. & Strande, C.S. (1973). A new micromethod for determination of protein in cerebrospinal fluid and urine. Clin. Chem. 19, 1265-1267.

Platman, S.R., Rohrlich, J., & Fieve, R.R. (1968). Absorption and excretion of lithium in manic-depressive disease. Dis. Nerv. System. XXIX, 733-738.

Prockop, L.D. & Marcus, D.J. (1972). Cerebrospinal fluid lithium: passive transfer kinetics. Life Sci. 11, 859-868.

Reed, D.J. & Yen, M.H. (1978). The role of the cat choroid plexus in regulating cerebrospinal fluid magnesium. J. Physiol. (in press).

Schou, M. (1958). Distribution between serum and tissues. Acta. Pharmacol. Toxicol. 15, 115-124.

Schou, M., Juel-Nielsen, N., Stromgreen, E., & Voldby, H. (1954). The treatment of manic psychoses by the administration of lithium salts. J. Neurosurg. Psychiat. 17, 250-260.

Watanabe, S., Taguchi, K., Ebara, T., Iguchi, K., & Otsuki, S. (1973). Lithium concentration in cerebrospinal fluid of affective psychotic patients treated with lithium carbonate and its clinical response. Folia. Psychiatrica et Neurologica 27, 299-303.

Whittam, R. (1962). Directional effects of alkali metal ions on adenosine triphosphate hydrolysis in erythrocyte ghosts. Nature 196, 134-136.

Wraae, O. (1978). The pharmacokinetics of lithium in the brain, cerebrospinal fluid and serum of the rat. In Lithium in Medical Practice. ed., Johnson, F.N. & Johnson, S. pp. 225-228. MIP Press: Lancaster.

Wright, E.M. (1972). Mechanisms of ion transport across the choroid plexus. J. Physiol. 226, 525-571.

Zerahn, K. (1955). Studies on the active transport of lithium in the isolated frog skin. Acta. Physiol. Scand. 33, 347-358.

PART TWO

THE EFFECT OF LITHIUM ON ELECTROLYTE TRANSPORT BY THE IN SITU CHOROID PLEXUS OF THE CAT

INTRODUCTION

The mechanisms underlying the therapeutic usefulness of lithium in mania are not well known. Investigations into the pharmacological action of lithium have been in two major areas, biogenic amines and electrolytes. A number of factors account for the growing interest in electrolyte metabolism in psychiatry. Electrolyte metabolism directly affects neural activity. Thus, electrolytes such as sodium and potassium are known to be essential for the transmission of electrical impulses within the nervous system and for the maintenance of resting potentials across cell membranes. Electrolyte metabolism may indirectly change brain function through its influence on neurotransmitters (Bogdanski & Brodie, 1966; Horst, Kopin, & Ramey, 1968). It is well established that ions are involved in many enzymatic processes including the storage, release, transport and activation of neurochemical modulators. For example, magnesium ions activate many enzyme systems, stabilize the organization of macromolecules and chelate many organic complexes.

A similarity of physical and chemical properties among lithium, sodium, potassium and magnesium has been reported. Lithium has a charge equal to that of sodium or potassium and an ionic radius similar to that of magnesium. Schou (1973) has commented that "it is indeed likely that the partial similarity of lithium to these biologically important cations may account for most of the biochemical and physiological effects of lithium salts". Gibbons (1960), Coppen & Shaw (1963);

Hullin, Swinscoe, McDonald, & Dransfield, (1968); and Aronoff, Evans & Durell., (1971); all reported that sodium excretion increased following the commencement of lithium treatment. Baer, Kassir & Fieve., (1970); Ho, Gershon & Pinkney, (1970); and Birch & Jenner (1973) found that chronic administration of lithium to rats results in a significant decrease in brain sodium concentration. Nielsen (1964) has demonstrated that serum magnesium levels are elevated following lithium administration to manic patients. Birch & Jenner (1973) observed that lithium routinely reduces brain magnesium and significantly increase plasma magnesium. Lithium treatment of rats increased both serum and urinary magnesium levels (Aronoff, et al., 1971; Andreoli, Villani & Brambilla., 1972 and Mellerup, Plenge & Rafaelsen., 1973). Therefore, the effect of lithium on the metabolism of electrolytes in the C.N.S. may be important in terms of understanding the action of lithium.

It is well established that the choroid plexus is a major site of c.s.f. production and also participates in regulating the composition of the c.s.f.. The in situ isolated choroid plexus preparation developed by miner & Reed (1972) was used in the present study to investigate the effect of varying the lithium concentration in plasma and in c.s.f. on the concentration of other electrolytes in the c.s.f. The results suggest that lithium has potassium-like effects in that it stimulates the rate ^{22}Na transport from blood to c.s.f. and of lithium from c.s.f. to blood. Lithium may be transported to a limited extent from blood to c.s.f. via the sodium transport system responsible for c.s.f. secretion.

METHODS and MATERIALS

The experimental preparation, c.s.f. collection procedures, sample analysis and calculations are described in the preceding paper (part one). Radioactive $^{22}NaCl$ (375 mCi/mg) was purchased from New England Nuclear. The rate of infusion was varied as necessary to attain a constant level of ^{22}Na in plasma when that was desired. The concentration of lithium and potassium in the chamber fluid was altered by interchanging NaCl and LiCl or NaCl and KCl in the artificial c.s.f.. The ouabain solution (10^{-5}M or 10^{-3}M) was prepared by adding ouabain to the artificial c.s.f. that contained either lithium (1.5 m-equiv/l.) or potassium (6.6 m-equiv/l.). Magnesium was determined by diluting 10 µl of chamber fluid (1:1000) or one ml of the plasma supernatant prepared as described in the preceding paper (1:10) with La-Sr solution (1.8×10^{-2}M La_2O_3 and 1.1×10^{-4}M $SrCl_2 \cdot 6\ H_2O$). Magnesium was measured on a model 305 A Perkin-Elmer Atomic Absorption Spectrophotometer. The radioactivity of ^{22}Na in plasma and c.s.f. was counted with a Nuclear-Chicago liquid-scintillation counter.

Experimental procedures: Four series of experiments were performed in this study.

First experiment: radioactive ^{22}Na was infused intravenously and a series of blood samples and chamber fluid was obtained at 30 min intervals.

Second experiment: LiCl solution was infused intravenously for 4 hr and a steady state concentration of ^{22}Na was reached after about 2 hr

Blood samples and chamber fluid were removed every 30 min.

Third experiment:artificial c.s.f. that contained 1.5 m-equiv/1. of LiCl or 6.6 m-equiv/1. of KCl was added to the chamber when a steady-state concentration of ^{22}Na was attained at 2 hr.

Fourth experiment:Ouabain (10^{-5}M or 10^{-3}M) was added to the lithium (1.5 m-equiv/1.) or potassium (6.6 m-equiv/1.) artificial c.s.f. and placed in the chamber when the steady-state for ^{22}Na was attained. Chamber fluid was collected every 30 min for 2 hr.

RESULTS

The average rate of fluid accumulation and the mean protein concentration in the new c.s.f. were similar to those described in the preceding paper, namely, 0.35 to 0.47 µl/min/mg tissue and 0.064 to 1.462 g/100 ml.

The effects of lithium infusion on the concentration of magnesium in plasma and chamber fluid are shown in Fig. 8. The concentration of magnesium in plasma was significantly increased ($P<0.01$) when the lithium concentration in the plasma was 2.7 m-equiv/l. or more, while chamber fluid magnesium was not affected. These results confirm the data reported by Nielsen (1964); Mellerup et al., (1973) and Birch & Jenner (1973). They are also consistent with the results of a previous study (Reed & Yen, 1978) of magnesium regulation by the choroid plexus in which the plasma magnesium concentration was varied with only a small effect on c.s.f. magnesium.

The ratio of chamber fluid ^{22}Na to plasma ^{22}Na vs. time is shown in Fig. 9. The ratio is greater than unity after 90 min. It is well established that there is a small concentration gradient for sodium between c.s.f. and plasma (Ames, Sakanoue, & Endo, 1964 and Cserr,1975).

The time course of ^{22}Na content of chamber fluid at 30 min intervals is shown in Fig. 10. The total amount of ^{22}Na in the chamber fluid was significantly increased from 625 cpm to 1365 cpm when lithium was added to the chamber and from 935 cpm to 1575 cpm with the addition of potassium ($P<0.01$). There is no difference in ^{22}Na activity

Fig. 8. Effects of lithium infusion on magnesium concentration in plasma and c.s.f. Each point on the graph represents the mean value of one 30 min collection of chamber fluid. The bar for each point represents the s.e.m. (n=5), calculated by Student's t test. *:$P< 0.05$. Plasma (o), Chamber fluid (o).

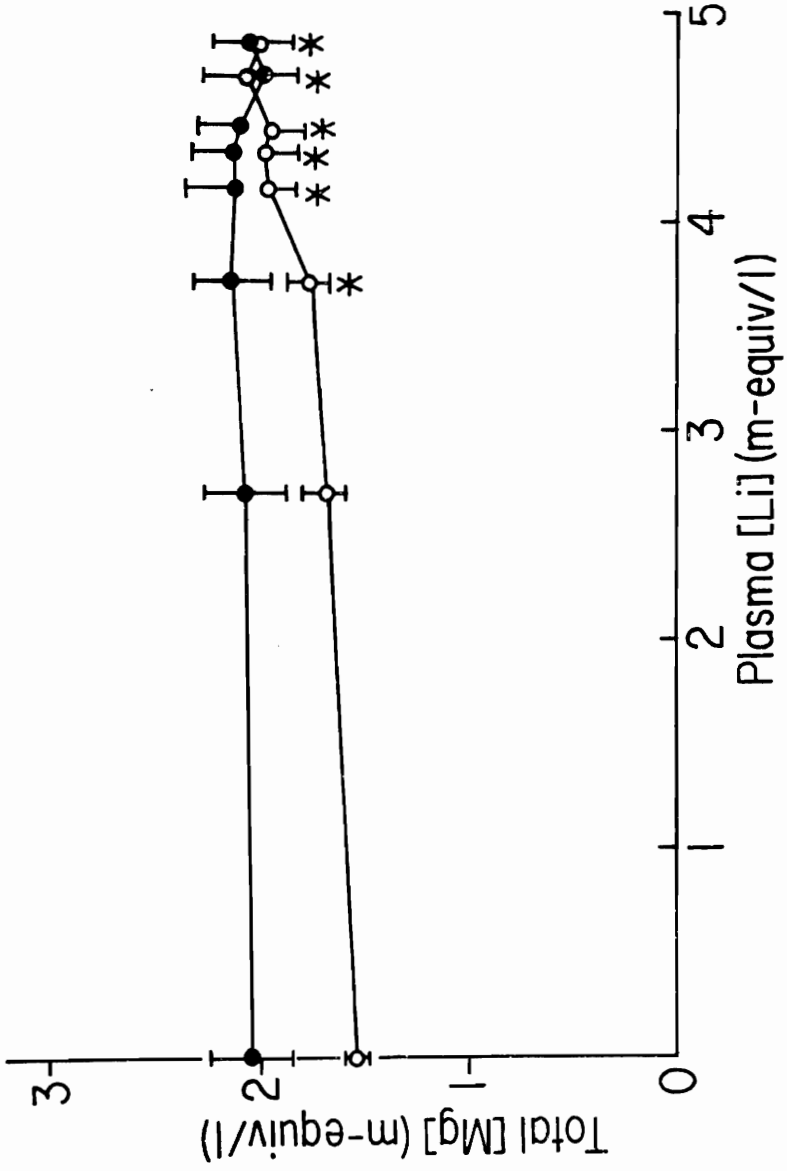

Total [Mg] (m-equiv/l)
3
2
1
0
1
2
3
4
5
Plasma [Li] (m-equiv/l)
*
*
*
*
*
*

Fig. 9. Chamber fluid to plasma ratio of ^{22}Na as a function of time after the start of infusion of $^{22}NaCl$ solution. The bar for each point represents the s.e.m., (n=7), calculated by student's t test. * ratios significantly different from one ($P<0.05$).

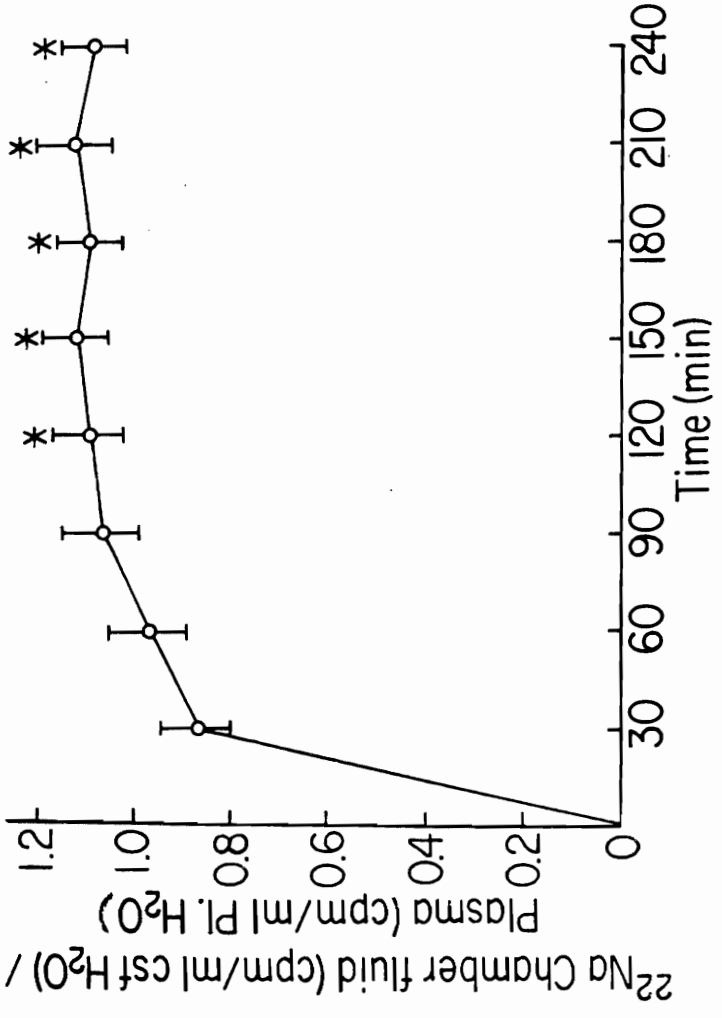
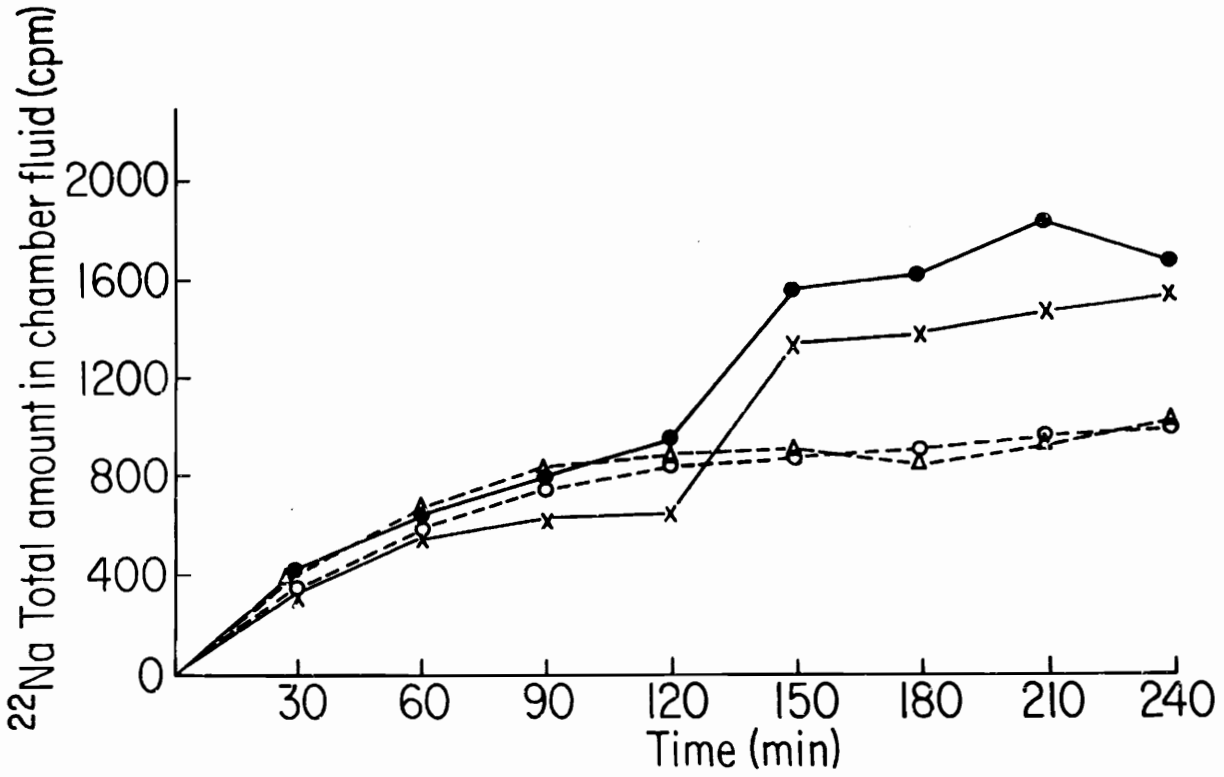

^{22}Na Chamber fluid (cpm/ml csf H_2O) /
Plasma (cpm/ml Pl. H_2O)
1.2
1.0
0.8
0.6
0.4
0.2
0
30
60
90
120
150
180
210
240
Time (min)
*
*
*
*
*

Fig. 10. Total amount of ^{22}Na in the chamber fluid vs. time. Starting at the 120 min period 50 µl of lithium (1.5 m-equiv/l.) or high-potassium (6.6 m-equiv/l.) artificial c.s.f. was added to the chamber. Each point on the graph represents the average of 5 animals. Control (0), 50 µl of normal artificial c.s.f. (Δ), high-potassium (●) and lithium (X).


^{22}Na Total amount in chamber fluid (cpm)
2000
1600
1200
800
400
0
30
60
90
120
150
180
210
240
Time (min)

in the chamber fluid between the controls and the experimental group in which 50 µl of normal artificial c.s.f. was added to the chamber. The rate of c.s.f. secretion does not change when either lithium or high-potassium is added to the chamber.

The effects of potassium and ouabain on ^{22}Na transport are shown in Fig. 11. When potassium (6.6 m-equiv/1.) was added to the chamber, the rate of ^{22}Na transport from blood to c.s.f. increased from 100 to 180% of the value at 120 min. However, when ouabain ($10^{-5}M$) was added in combination with potassium the rate of ^{22}Na transport increased 205%. Thus, a slight additional stimulation of ^{22}Na transport also occurs when ouabain ($10^{-5}M$) in addition to high-potasium artificial c.s.f. is added. However, $10^{-3}M$ ouabain had a significantly inhibitory effect since both ^{22}Na transport from blood to c.s.f. and c.s.f. secretory rate are reduced.

The effects of lithium and ouabain on ^{22}Na transport are summarized in Fig. 12. When lithium (1.5 m-equiv/1.) was added to the chamber, the rate of ^{22}Na transport from blood to c.s.f. increased from 100 to 240% of the value at 120 min and was still increasing after 240 min. However, when ouabain ($10^{-5}M$) was added in combination with lithium (1.5 m-equiv/1.), the ^{22}Na transport increased only to 180% of the 120 min value and was maintained at this level throughout the experiment. When ouabain was increased to $10^{-3}M$ the rate of ^{22}Na transport into the chamber was significantly inhibited and the rate of c.s.f. secretion was reduced (26%).

The total amount of ^{22}Na in the chamber fluid is plotted as a function of time in Fig. 13. The plasma lithium concentration was altered by infusion of LiCl solution starting at 120 min. The total

Fig. 11. The ^{22}Na content of the chamber fluid expressed as a percent of the 120 min value as a function of time. At 120 min high-potassium (6.6 m-equiv/l) fluid was added to the chamber with or without ouabain (10^{-5}M or 10^{-3}M). Control (o), high-potassium (▽), high-potassium + ouabain (10^{-5}M) (●) and high-potassium +ouabain (10^{-3}M) (X). Each point represents the average of 5 animals.

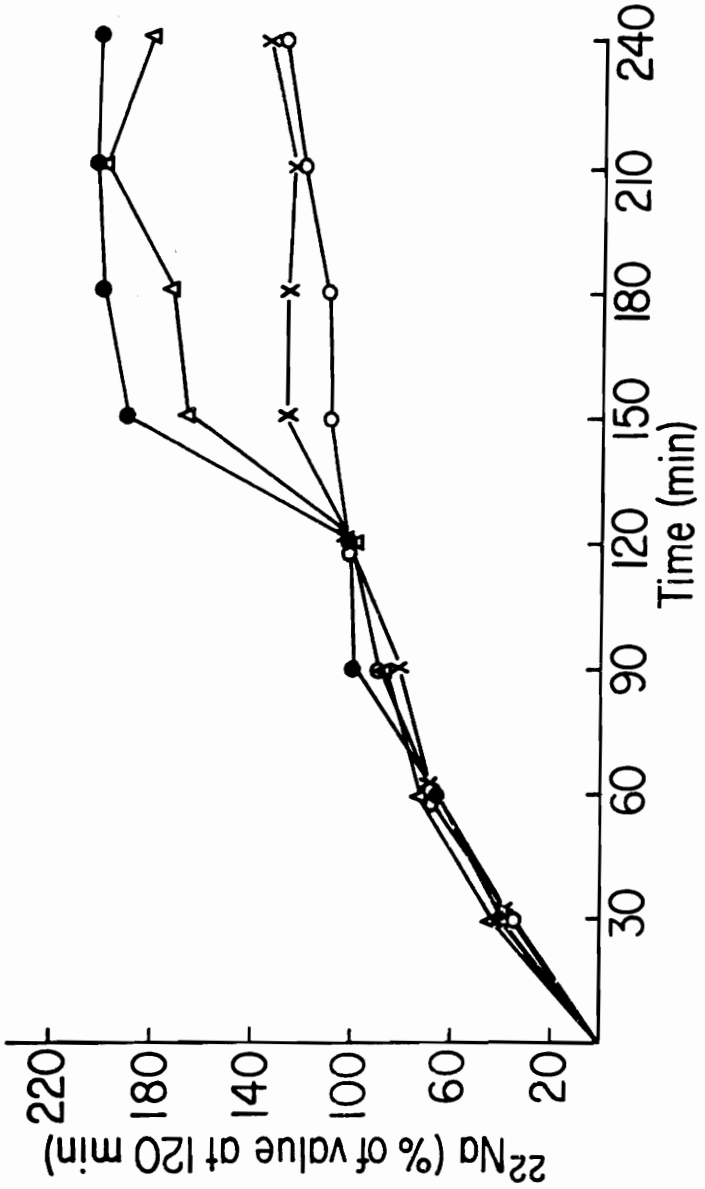

^{22}Na (% of value at 120 min)
220
180
140
100
60
20
30
60
90
120
150
180
210
240
Time (min)

Fig. 12. The ^{22}Na content of the chamber fluid expressed as a percent of the 120 min value as a function of time. At 120 min lithium (1.5 m-equiv/1.) fluid was added to the chamber with or without ouabain (10^{-5}M or 10^{-3}M). Control (o), lithium (Δ), lithium + ouabain (10^{-5}M) (●) and lithium + ouabain (10^{-3}M) (X). Each point represents the average of 5 animals.

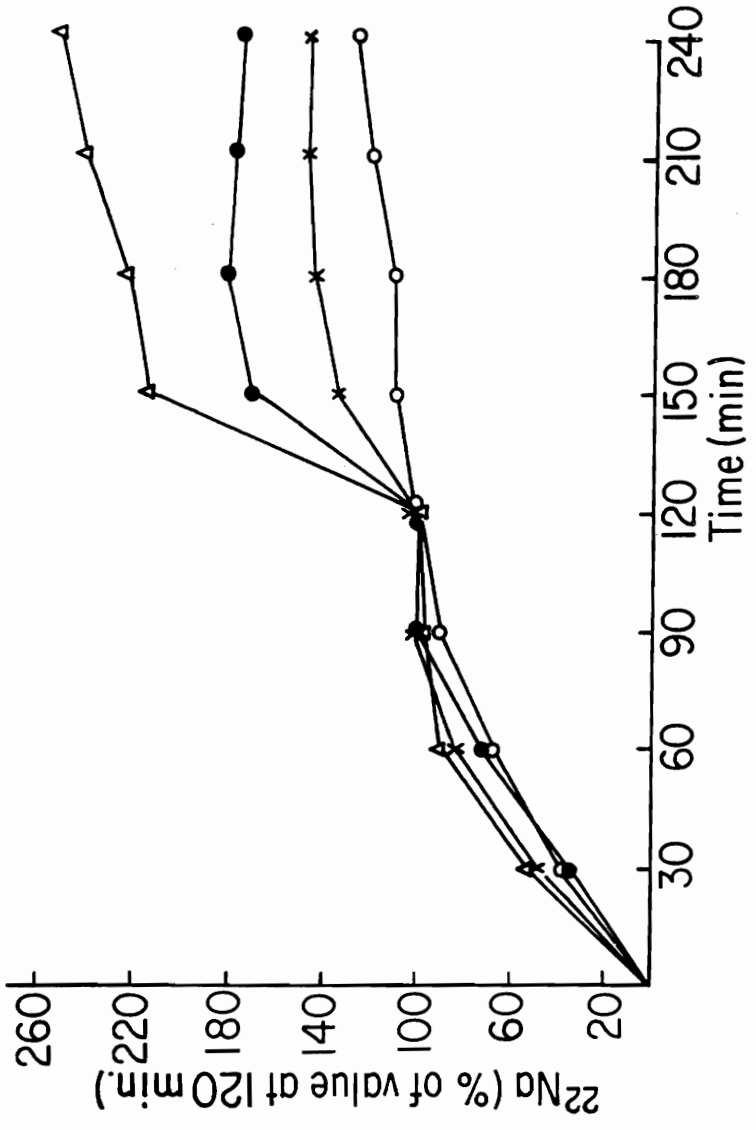
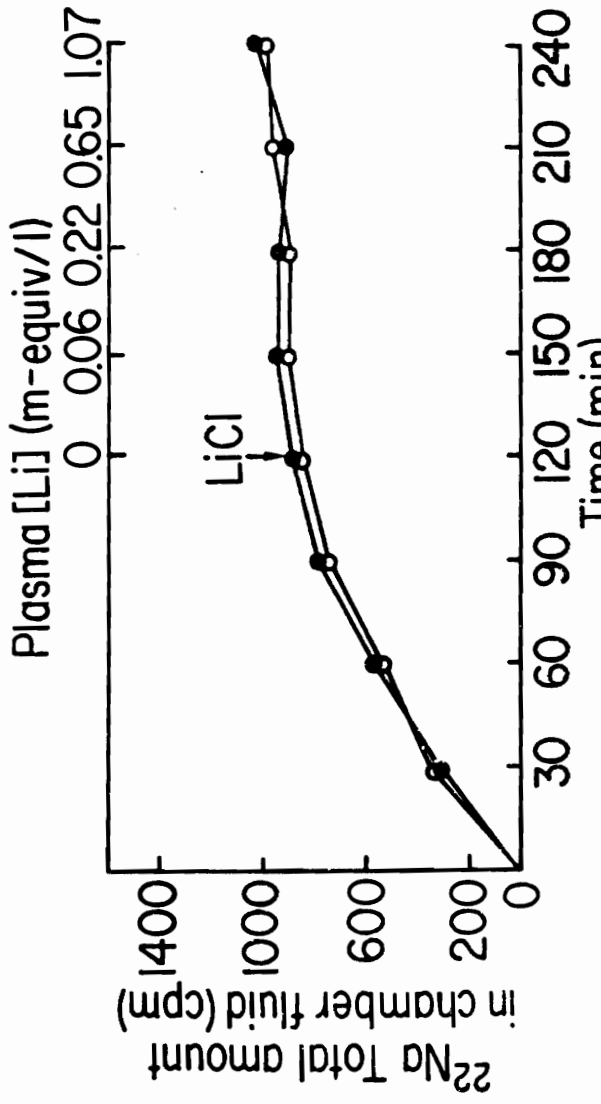

260
220
180
140
100
60
20
^{22}Na (% of value at 120 min.)
30
60
90
120
150
180
210
240
Time (min)

Fig. 13. The total amount of ^{22}Na in the chamber versus time. LiCl (154 mM) was added to the i.v. infusion fluid starting at 120 min. Lithium concentration in plasma ranged from 0.06 to 1.07 m-equiv/l. Each point represents the average of 4 animals. ^{22}Na infusion (o) and ^{22}Na + LiCl infusion (●).


Plasma [Li] (m-equiv/l)
0 0.06 0.22 0.65 1.07
LiCl
^{22}Na Total amount
in chamber fluid (cpm)
0 200 600 1000 1400
30 60 90 120 150 180 210 240
Time (min)

amount of ^{22}Na in chamber fluid was not affected by lithium ($P>0.05$) when the plasma lithium level was varied from subtherapeutic to therapeutic values in man (0.06 to 1.07 m-equiv/l.).

DISCUSSION

Lithium infusion significantly increases plasma magnesium concentration. This is consistent with a number of reports which have shown that lithium treatment is accompanied by increased plasma magnesium concentrations (Nielsen, 1964; Bunney, Goodwin, Davis & Fawcett, 1968; Aronoff et al., 1971; Andreoli, et al., 1972 Birch & Jenner, 1973; Mellerup, et al., 1973). Aronoff et al. (1971) reported that the serum magnesium concentration increased in all but one of their lithium-treated patients. These increases were accompanied by elevated urinary levels of magneisum. Birch & Jenner (1973) reported that in rats lithium apparently leads to a reduction in brain magnesium at the same time that plasma magnesium levels are increased. In the present study the magnesium concentration in chamber fluid was not significantly changed when plasma magnesium was increased by lithium infusion. These data confirm the results of Reed & Yen (1978) who reported that when the plasma magnesium concentration is varied the chamber fluid magnesium remains relatively constant.

The appearance of ^{24}Na in c.s.f. following its intravenous administration has been studied in dogs by Wang (1948), Olsen & Rudolph (1955) and Fishman (1959). The rate of exchange of sodium between plasma and c.s.f. varied depending upon the site at which the c.s.f. is sampled. Sweet, Selverstone, Solomon & Bakay, (1949) and Davson, H. (1958) studied the exchange of ^{24}Na between plasma and the lateral ventricle, subarachnoid space, lumbar sac and found that the ventri-

cular fluid came into equilibrium with the plasma much more rapidly than did the fluid in the subarachnoid spaces. The authors suggest that ^{24}Na probably entered the c.s.f. largely by way of the choroid plexuses. The ratio of ^{22}Na in c.s.f. to that of plasma shown in Fig. 9 demonstrates that the radioactive sodium in chamber fluid reaches equilibrium with plasma very rapidly, which is to be expected since the chamber fluid is derived from plasma during the 30 min collection period. Also the chamber fluid/plasma ratio of ^{22}Na is larger than unity. This indicates a slight concentration gradient of ^{22}Na between chamber fluid and plasma. The active transport of sodium across the choroid plexus has long been implicated as the driving force for c.s.f. secretion. To cite one example, Vates, Bonting & Oppelt, (1964) found a quantitative correlation between the inhibition of c.s.f. formation by cardiac glycoside and the activity of the Na-K ATPase in the choroid plexus. This suggests that an active transport process is involved in maintaining the sodium gradient between c.s.f. and plasma, and in c.s.f. secretion.

The entry of radioactive sodium into the c.s.f. from blood during ventriculocisternal perfusion is thought to consist of two components, namely, fast and slow (Davson & Pollay, 1963). Davson & Segal (1970) suggested that the fast component of entry was probably due to transport directly from blood to c.s.f. across the choroid plexus; the slow component was thought to be due to transport from blood to brain and thence to c.s.f.. Also, it has been demonstrated that the fast component of sodium entry is due to a sodium pump system within the choroid plexus (Davson & Segal, 1970; Wright, 1972). The isolated choroid plexus used in the present studies is uniquely suited for inves-

tigating the fast component of sodium entry without the complicating presence of the slow component. On the basis of the observations cited above, one would expect that the ^{22}Na in c.s.f. should rapidly reach equilibrium with plasma. This was found to be the case.

Large changes in plasma potassium concentration cause relatively small alterations in c.s.f. potassium concentration (Bekaert & Demeester, 1951; Domer & Whitcomb, 1964; Bradbury & Davson, 1965; Ames, Higashi & Nesbett, 1965; Husted & Reed, 1976). It has been demonstrated by Husted & Reed (1976) that active transport of potassium from c.s.f. to plasma occurs and that the process is under the control of a mechanism that senses c.s.f. potassium concentration. Bradbury & Stulcova (1970) and Johanson, Reed & Woodbury, (1974) have found evidence that the choroid plexus sodium pump is situated at the apical (c.s.f. — facing) membrane and this hypothesis is supported by the demonstration of ouabain binding to the apical membrane of the frog choroid plexus (Quinton, Wright & Tormey, 1973). Recently, Hesketh (1977) reported that in incubated choroid plexus of the rabbit the ATPase activity is sensitive to the potassium concentration. In the present experiments it was shown (Fig. 10) that potassium significantly increases sodium entry from blood to c.s.f. when high-potassium artificial c.s.f is present on the c.s.f. side. This suggests that the increased sodium entry in response to elevated potassium in the chamber fluid is due to activation of the Na-K exchange pump or perhaps to stimulation of the sodium pump responsible for c.s.f. secretion. Although the calculated concentration of ^{22}Na in the newly formed fluid is significantly increased, the rate of c.s.f. secretion is not affected when high-potassium is added to the chamber. This indicates that the increase in

^{22}Na in the chamber fluid is not caused by an increase in the rate of fluid production but is due to the stimulation of the Na-K exchange transport by the high potassium. Husted & Reed (1976) also found that the rate of c.s.f. secretion is not changed when potassium concentration in plasma or c.s.f. is altered.

Ouabain was used to ascertain whether the stimulatory effect of potassium on ^{22}Na entry from blood to c.s.f. was due to stimulation of a sodium pump. A dose of ouabain ($10^{-5}M$) similar to those used in several ventriculocisternal perfusion experiments (Vates et al., 1964; Cserr, 1965; Pollay, 1975), in situ choroid plexus preparations (Welch, 1963) or in vitro choroid plexus incubation studies (Wright, 1972) was used in these experiments. Ouabain ($10^{-5}M$) slightly potentiated instead of inhibiting the stimulatory effect of high-potassium on ^{22}Na entry (Fig. 11.) These observations are similar to the data presented by Oppelt, Patlack & Rall, (1964) from a ventriculocisternal perfusion study, in which a stimulatory effect on c.s.f. production was demonstrated with a low (10^{-8}-$10^{-12}M$) concentration of ouabain. The concentration of ouabain at the site of action in the present study may have been lower than the concentration in the solution ($10^{-5}M$) and may have been as low as the concentration of ouabain (10^{-8}-$10^{-12}M$) used by Oppelt (1964). However, when the ouabain concentration of the fluid added to the chamber was increased from $10^{-5}M$ to $10^{-3}M$, there was a significant inhibition of the stimulatory effect of high-potassium on the rate of ^{22}Na transport form blood to c.s.f. and on the rate of c.s.f. secretion. These inhibitory effects are less than those cited above when the same doses were used in both in vitro or in vivo studies. This may be due to inadequate stirring of the chamber fluid. The choroid

plexus is a ciliated epithelium whose cilia beat at a rapid rate. It has been postulated that the actions of the cilia are responsible in part for circulating or mixing the c.s.f.. In the case of the present studies the composition of the layer of c.s.f. adjacent to the choroid plexus in the chamber fluid may be significantly different from that in the bulk of the chamber fluid if the action of the cilia are inadequate to provide good mixing of the chamber contents with the newly secreted fluid. The fact that the choroid plexus is continuously secreting new ouabain free fluid at the cell surface to form a buffer between the ouabain solution and the cell would also be expected to reduce the effectiveness of the ouabain added to the chamber.

Potassium may interfere with the action of ouabain. Godlman, Coltart, Friedman, Nola, Berke, Schweizer & Harrison, (1973) showed that hyperkalemia alters the inotropic effects of digoxin by decreasing the binding of digoxin by a microsomal cell fraction containing Na-K ATPase activity. Akera, Brody, So. Tobin & Baskin, (1974) also presented evidence that the effects of potassium are consistent with a conformational change in the ATPase such that the binding sites for ouabain become less accessible. These experiments provide further evidence that a high concentration of potassium in the c.s.f. stimulates teh transport of sodium across the choroid plexus by a ouabain-sensitive process.

Birch & Jenner (1973) showed that lithium in plasma acts to decrease the brain sodium concentration and suggested that lithium may activate Na-K ATPase at the potassium sensitive site. The data shown in Fig. 10 demonstrate that lithium can significantly increase the rate of ^{22}Na transport from blood to c.s.f. in a manner similar to potass-

ium when lithium is added directly to the chamber. The calculated ^{22}Na concentration in the newly formed fluid was significantly increased while the rate of c.s.f. secretion was not affected when lithium was present in the chamber. This indicates that the increase in ^{22}Na in the chamber fluid is not caused by an increase in the rate of fluid production, but rather is due to the lithium stimulating the transport of ^{22}Na from blood to c.s.f.. In vitro and in vivo studies indicate that the passive diffusion of lithium ions resembles that of sodium ions and that lithium can substitute for both sodium and potassium in some active transport mechanisms (Keynes & Swan, 1959; McConaghey & Maizels, 1962). Sjodin & Beauge (1968) suggested that lithium ions directly stimulate a sodium pump mechanism in skeletal muscle that results in increased sodium efflux and in later experiments they confirmed this suggestion (Beauge & Ortiz, 1970). Glen, Bradbury, & Wilson, (1972) found that 3 mM lithium stimulates sodium efflux from erythrocytes. Confirmation of this finding has come from recent work of Hesketh (1977) who reported that ventriculocisternal perfusion studies in the rabbit show that lithium increased the rate of sodium entry into the c.s.f.. Fig. 10 shows that lithium significantly increases the rate of ^{22}Na transport in a manner similar to potassium. Ouabain (10^{-3}M) significantly inhibits the rate of ^{22}Na entry into the c.s.f. as shown in Fig. 12. On the basis of the present studies it appears that lithium has an effect similar to that of potassium to stimulate the sodium pump.

Fig. 13. shows that when lithium is administered intravenously there is no effect on ^{22}Na transport. These results are different from those of Hesketh (1977) who reported that when lithium was pre-

sent on the blood side there was a marked decrease in the fast component of sodium entry. However, Smith & Balagura (1972) found that when lithium is administered orally to rats there no effect on sodium concentration in the c.s.f. This discrepancy may be due to the difference in experimental preparation and/or species difference.

Reports in the literature provide strong evidence that active sodium pumping produces the osmotic gradient which is responsible for c.s.f. production (Diamond, 1965; Diamond & Bossert, 1967; 1968; Segal & Pollay, 1977). The present observations show that both lithium and an increase in potassium significantly increases the rate of ^{22}Na transport from blood to c.s.f., but had no effect on the rate of c.s.f. secretion. The fact that in neither case did the rate of c.s.f. secretion increase strongly implies that the sodium pump responsible for c.s.f. secretion was not responsible for the increased rate of entry of ^{22}Na into the chamber fluid. Since it has been demonstrated previously in this preparation that potassium is actively removed from the c.s.f., probably by an Na-K exchanges mechanism, it seems likely that there are functionally two sodium pump transport systems in the choroid plexus that move sodium from blood to c.s.f., one primarily responsible for c.s.f. secretion and one involved in potassium regulation via sodium-potassium exchange. The presence of two sodium pumps in other tissues has been postulated by Hoffman & Kregenow (1966) for the red blood cell and Whittembury & Proverbio (1970) for the renal cortex. The presence of two sodium pumps in the choroid plexus would explain the results presented in this study.

REFERENCES

Akera, T., Brody, T.M., So, R. H-M., Tobin, T. & Baskin, S.I. (1974). Factors and agents that influence cardiac glycosides-Na, K ATPase interaction. Ann. N.Y. Acad. Sci. 242, 617-634.

Ames, A., Sakanoue, M., & Endo, S. (1964). Na, K, Ca, Mg, and Cl concentrations in choroid plexus fluid and cisternal fluid compared with plasma ultrafiltrate. J. Neurophysiol. 27, 672-681.

Ames, A., Higashi, K. & Nesbett, F.B. (1965). Relation of potassium concentration in choroid plexus fluid to that in plasma. J. Physiol. 181, 506-515.

Andreoli, V.M., Villani, F., & Brambilla, G. (1972). Increased calcium and magnesium excretion induced by lithium carbonate. Psychopharmacologia. Berl., 25, 77 85.

Aronoff, M.S. Evens, R.G., & Durell J. (1971). Effect of lithium salts on electrolyte metabolism. J. Psychiat. Res. 8, 139-159.

Baer, L., Kassir, S., & Fieve, R.R. (1970). Lithium induced changes in electrolyte balance and tissue electrolytes. Psychopharmacologia. 17, 216-224.

Beauge, L.A. & Ortiz, O. (1970). Sodium efflux in frog skeletal muscle. Biochem. Biophys. Acta. 219, 479-483.

Bekaert, J., & Demeester, G. (1951). The influence of the infusion of potassium chloride on the cerebrospinal fluid concentration of potassium. Archs. int. Physiol. 59, 393-395.

Birch, N.J. & Jenner, F.A. (1973). The distribution of lithium and its effects on the distribution and excretion of other ions in the rat. Brit. J. Pharmacol. 47, 586-594

Bogdanski, D.F. & Brodie, B.B. (1966). Role of sodium and potassium ions in storage of norepinephrine by sympathetic nerve endings. Life Sci. 5, 1563-1569.

Bradbury, M.W.B. & Davson, H. (1965), The transport of potassium between blood, cerebrospinal fluid and brain. J. Physiol. 181, 151-174.

Bradbury, M.W.B. & Stulcova, B. (1970). Efflux mechanism contributing to the stability of the potassium concentration in cerebrospinal fluid. J. Physio. 208, 415-430.

Bunney, W.E., Goodwin, F.E., Davis, J.M., & Fawcett, J.A. (1968). A Behaviour-biochemical study of lithium treatment. Am. J. Psychiat. 125, 499-512.

Coppen, A. & Shaw, D.M. (1963). Mineral metabolism in melancholia. Brit. Med. J. 2, 1439-1444.

Cserr, H.F. (1965). Potassium exchange between cerebrospinal fluid, plasma and brain. Am. J. Physiol. 209, 1219-1226.

Cserr, H.F. (1975). Physiology of the choroid plexus. In The Choroid Plexus in Health and Disease. ed., Netsky, M.G. & Shuangshoti, S. pp. 175-195. University Press:Virginia.

Davson, H. (1958). Some aspects of the relationship between the cerebrospinal fluid and the central nerve system. In The Cerebrospinal Fluid. ed., Wolstenholme, G.E. W.& Oconnor, C.M. pp. 190-208. Little Brown: Boston.

Davson, H. & Pollay, M. (1963). The turnover of ^{22}Na in the cerebrospinal fluid and its bearing on the blood-brain barrier. J. Physiol. 167, 247-255.

Davson, H. & Segal, M.B.(1970). The effects of some inhibitors and accelerators of sodium transport on the turnover of ^{22}Na in the cerebrospinal and the brain. J. Physiol. 209, 131-153.

Diamond, J.M.(1965). The mechanism of isotonic water transport. Symp. Soc. Exp. Biol. 19, 329-348.

Diamond, J.M. & Bossert, W.H. (1967). Standing-gradient osmotic flow: A mechanism for coupling of water and solute transport in epithelia. J. Gen. Physiol. 50, 2061-2083

Diamond, J.M. & Bossert, W.H. (1968). Functional consequences of ultrastructural geometry in "backwards" fluid-transporting epithelia. J. Cell. Biol. 37, 694-702.

Domer, F.R. & Whitcomb, M. (1964). Studies of ^{42}K movement between the blood and the cerebrospinal fluid of cats. J. Pharmac. Exp. Ther. 145, 52-57.

Fishman, R.A. (1959). Factors influencing the exchange of sodium between plasma and cerebrospinal fluid. J. Clin. Invest. 38, 1698-1708.

Gibbons, J.L. (1960). Total body sodium and potassium in depressive illness. Clin. Sci. 19, 133-138.

Glen, A.I.M., Bradbury, M.W.B., & Wilson, J. (1972). Stimulation of sodium pump in the red blood cell by lithium and potassium. Nature (London), 239 399-401.

Goldman, R.H., Coltart, D.J., Firedman, J.P., Nola, G.T., Berke, D.K.,

Foldman, R.H., Coltart, D.J., Friedman, J.P., Nola, G.T., Berke, D.K. Schweizer, E., & Harrison, D.C. (1973). The inotropic effects in the intact animal. Circulation XLVIII, 830-838.

Hesketh, J.E. (1977). Effects of potassium and lithium on sodium transport form blood to cerebrospinal fluid. J. Neurochem. 28, 597-603.

Ho, A.K.S., Gershon, S., & Pinckney, L. (1970). The effects of acute and prolonged lithium treatment on the distribution of electrolytes, potassium and sodium. Archs, int. Pharmacodyn. Ther. 186, 54-65.

Hoffman, J.F. & Kregenow, F.M. (1966). The characterization of new energy dependent cation transport processes in red blood cell. Ann. N.Y. Acad. Sci. 137, 566-576.

Horst, W.D., Kopin, I.J., & Ramey, E.R. (1968). Influence of sodium and calcium on norephinephrine uptake by isolated perfused rat hearts. Am. J. Physiol. 215, 817-822.

Hullin, R.P., Swinscoe, J.C., McDonald, R., & Dransfield, G.A. (1968). Metabolic balance studies on the effect of lithium salts in manic-depressive psychosis. Brit. J. Psychiat. 114, 1561-1573

Husted, R.F. & Reed, D.J. (1976). Regulation of cerebrospinal fluid potassium by the cat choroid plexus. J. Physiol. 259, 213-221.

Johanson, C.E., Reed, D.J., & Woodbury, D.M. (1974). Active transport of sodium and potassium by the choroid plexus of the rat. J. Physiol. 241, 359-372.

Keynes, R.D. & Swan, R.C. (1959). The permeability of frog muscle fibers to lithium ions. J. Physiol. 47, 626-638.

McConaghey, P.D. & Maizels, M. (1962). Cation exchanges of lactose-treated human red cells. J. Physiol. 162, 485-509.

Miner, L.C. & Reed, D.J. (1972). Composition of fluid obtained from choroid plexus tissue isolated in a chamber in situ. J. Physiol. 227, 127-139.

Mellerup, E.T. Plenge, P., & Rafaelsen, O.T. (1973) Lithium effects on magnesium metabolism in rats. Int. Pharmacopsychiat. 8, 178-183.

Nielsen, J. (1964). Serum and erythrocyte magnesium in patients with manic states during lithium treatment. Acta. Psychiat. Scand. 40, 190-196.

Olsen, N.S. & Rudolph, G.G. (1955). Transfer of sodium and bromide ions between blood, cerebrospinal fluid and brain tissue. Am. J. Physiol. 183, 427-432.

Oppelt, W.W., Patlak, C.S., & Rall, D.P. (1964). Effect of certain drugs on cerebrospinal fluid production in the dog. Am. J. Physiol. 206, 247-250.

Pollay, M. (1975). Formation of cerebrospinal fluid. J. Neurosurg. 42, 665-673.

Quinton, P.M., Wright, E.M., & Tormey, J. McD. (1973). Localization of sodium pumps in the choroid plexus epithelium. J. Cell Biol. 58, 724-730.

Reed, D.J. & Yen M.H. (1978). The role of the cat choroid plexus in regulating cerebrospinal fluid magnesium. J. Physiol. (in press).

Schou, M. (1973). Possible mechanisms of action of lithium salts: approaches and perspectives. Biochem. Soc. Trans. 1, 81-87.

Segal, M.B. & Pollay, M. (1977). The secretion of cerebrospinal fluid. Exp. Eye. Res. Suppl. 127-148.

Sjodin, R.A. & Beauge, L.A. (1968). Strophanthidin sensitive components of potassium and sodium movements in skeletal muscle as influenced by the internal sodium concentration. J. Gen, Physiol. 52, 389-407).

Smith, D.F. & Balagura, S. (1972). The effect of lithium chloride on the electrolyte composition of cerebrospinal fluid of the rat. Physiol. Behavior 9, 261-262.

Sweet, W.H., Selverstone, B., Solomon, A., & Bakay, L. (1949). Studies of formation, diffusion and absorption of constituents of cerebrospinal fluid in man. I. studies with ^{24}Na. J. Clin. Invest. 28, 814.

Vates, T.S., Bonting, S.L., & Oppelt, W.W. (1964). Na-K activated adenosine triphosphatase formation of cerebrospinal fluid in the cat. Am. J. Physiol. 206, 1165-1172.

Wang, J. (1948). Penetration of radioactive sodium and chloride into cerebrospinal fluid and aqueous humor. J. Gen. Physiol. 31, 259-268.

Welch, K. (1963). Secretion of cerebrospinal fluid by choroid plexus of the rabbit. Am. J. Physiol. 205, 617-623.

Wittembury, G. & Proverbio, F. (1970). Two modes of sodium extrusion in cells from guinea-pig kidney cortex slices. Pflugers Arch. 316, 1-25.

Wright, E.M. (1972). Mechanisms of ion transport across the choroid plexus. J. Physiol. 226, 525-571.

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Publications

Chow,S.Y., Chang, R.L. and Yen, M.H.: A comparison between the uptake of radioactive perchlorate and iodide by rat and guinea-pig thyroid glands. J. Endocr., 45, 1-8, (1969).

Chow, S.Y., Yen, M.H., Lin, A.I.L. and Chang, C.F.: Effect of sodium glycolate and sodium glycolate and sodium ferulate on renal functions in dog. Archives int. Pharmacodyn. et de Ther. 177, 90-99, (1969).

Yen, M.H., Chow, S.Y. and Chang, R.L.: Perchlorate and renal excretory functions. Chinese J. Physiol. 21, 205-214, (1973).

Yen, M.H., Mu, J.Y. and Yin, T.H.: Plasma inulin concentration in response to glucose given by different routes. Chinese J. Physiol. 21, 193-204- (1973).

Tsai, H.Y., Yen, M.H. and Chow, S.Y.: Comparative effects of domamine and diphenhydramine on cardiovascular and renal responses to histamine-induced shock in dogs. Chinese J. Physiol. 21,181-192, (1973).

Yen, M.H. and Chow, S.Y.: Effects of intravenous infusion of ouabain on respiration. European J. Pharmac. 28, 95-99, (1974).

Reed, D.J. and Yen, M.H.: Regulation of csf magnesium by the isolated choroid plexus in situ. J. Physiol. (1978) in press.

Abstracts

Yen, M.H. and Reed, D.J: The regulation of lithium in csf by isolated cat choroid plexus in situ. ASPET/SOT meeting (1978).